THE ESTIMATION OF UROBILIN AND UROBILINOGEN IN THE DUODENAL CONTENTS.

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In a study of diseases associated with evidence of increased blood destruction it is desirable to attempt to estimate the activity of hemolysis and to compare this with the probable capacity for blood regeneration. Many workers have used as an index of blood destruction the changes that occur in the contents of the bile, namely, the amount of bile pigment excreted, and especially the amount of urobilinogen and urobilin. Eppinger estimated the amount of urobilinogen and urobilin in the stools by means of the Charnas spectrophotometric method and stimulated interest in this manner of estimating blood destruction, particularly in pernicious anemia and hemolytic icterus. In this country a more simple procedure for making rough quantitative estimates of these substances has been devised by Wilbur and Addis. Robertson, making use of the method, has reported findings in every way comparable to those of Eppinger.

The theories advanced with regard to the fate of hemoglobin after hemolysis are not well substantiated. Recent investigations by Whipple and Hooper are especially important. Their experiments involved a great deal of careful study over a considerable period of time, and, of necessity, only on dogs. Their conclusions are in some respects iconoclastic. If further work substantiates their findings it may be necessary to change our conception of the factors concerned in the formation of bile pigment. These authors conclude, especially from their results regarding the influence of diet on bile-pigment production, that the disintegration of red blood cells is not the important factor in the production of bile pigments. They have made estimations chiefly of the amount of bilirubin. It will be interesting to learn of their results with respect to urobilin and urobilinogen, for in the clinical studies on the hemolytic anemias there is now considerable evidence from many observers that the amounts of these substances excreted are definitely increased. This increase, it is true, may be the result of impaired organ function as well as of blood destruction. The quantity of bilirubin does not, however, run parallel to the quantity of urobilin and urobilinogen in pathological conditions.

In our own studies we have made use of the modifications of the Wilbur and Addis methods recently devised and reported by

Schneider. The essential advance in technic is the application of the observations, not to stool extracts, but to the contents of the duodenum as collected by means of an Einhorn tube. In this liquid an amount of the biliary pigments and their derivatives sufficient for quantitative determinations is readily obtained in a short time. While an estimation of the amounts of these pigments in the duodenal contents at a given time cannot be regarded as an index of the total amount excreted in twenty-four hours, objections are also advanced with respect to the values obtained in the twenty-four-hour stool in which some proportion of the substances may be destroyed or changed in character. The values obtained by Schneider's method have been so definitely in accord with the clinical manifestations that there is little doubt of the existence of a relationship which it is to be hoped may be made clearer by further study. These values are also in accord with the results obtained from estimations on the stool. The technic we have used, which in all of its essentials is that described by Schneider, is presented in detail, in order that it may be readily understood and easily followed. We wish to express our indebtedness to the originator of this method for his personal interest in our work.

The Method of Obtaining the Duodenal Contents.*

The tube and the metal capsule employed are similar to those of the Einhorn duodenal tube, but experience has led to the use of a somewhat stiffer tube and a capsule which, though similar in shape, is slightly larger and heavier, weighing 6.4 gm. This model of capsule was recommended by Schneider. However, an ordinary Einhorn or a Rehfuss bucket may be used. For convenience in observing the contents of the tube a piece of glass tubing is inserted at its end.

In the preparation for the examination the patient is instructed to partake of no food for at least twelve hours, except perhaps a little tea or coffee without cream, and to take frequent sips of warm water up to the time of the examination. The nature and purpose of the test are also explained, as the patient's confidence and coöperation aid in the passage of the tube.

The passage of the duodenal tube is a simple procedure. The metal capsule is placed on the back of the tongue and the patient is directed to swallow hard several times in rapid succession. There is usually some difficulty as the capsule reaches the level of the cricoid, but this is overcome by deep breathing. After the capsule has passed this irritable zone, peristalsis carries it along without further discomfort to the patient and largely beyond his control. He merely swallows from time to time, taking a few sips of warm water and the capsule finally reaches the stomach and comes to rest at about 60 cm. from the incisor teeth.

* Described by Szlapka.

The patient is now made to lie on his right side, with the hips elevated eight or ten inches. The pyloric end of the stomach thus becomes more dependent, and gravity, aided by gastric peristalsis, causes the capsule to pass through into position in the duodenum—some 70 cm. from the incisors. In our experience this is accomplished in from fifteen minutes to an hour, usually in about forty-five minutes. At no time is it necessary to push the tube on its way. Pushing only tends to coil it up in the stomach and may even frustrate its passage into the duodenum.

With the patient on his side the end of the tube is allowed to hang well over the edge of the table. Gastric contents siphon out first, the siphon being started by the injection of a few cubic centimeters of warm water. As the capsule moves through the pylorus into the duodenum the fluid recovered becomes yellowish and finally a clear bile is obtained. It may vary in color from light yellow to chocolate brown. Pure duodenal fluid is faintly alkaline, clear, of quite uniform color and viscid; the foam is golden. The liquid must be alkaline. Its mere appearance is not a safe guide as to its identity, although with experience one may come to recognize it readily. The character of the fluid collected must be observed closely, as from time to time the pylorus permits the passage of gastric contents into the duodenum. This impure liquid may be detected by its change in color and its dull, cloudy, opalescent appearance, diminished viscidity, more rapid flow, and change in reaction to litmus or Congo red. It should, of course, be discarded.

Should the flow of bile become interrupted an unusual length of time, the injection of a little warm water into the tube or the taking of deep breaths by the patient, will help to reëstablish it. The application of suction is neither necessary nor advisable.

It is our custom to collect the duodenal contents in a small, amber-colored bottle, as air and light cause the rapid transformation of the urobilinogen into urobilin; 20 c.c. of liquid are necessary for the test, which should be made immediately.

Laboratory Technic.*

The duodenal contents is poured into a graduated cylinder as soon as it is brought to the laboratory, and its gross appearance noted. Normally it is a light, straw-colored, viscid fluid, and this is reported as normal yellow (N). The color may vary to dark yellow, brown, and chocolate. The dark-colored fluids always yield much bilirubin, but the color of the duodenal contents does not always indicate the amount of urobilinogen or urobilin present, as these derivatives of bilirubin are sometimes demonstrated in considerable amounts in normal yellow fluids. Occasionally, however, in cases other than pernicious anemia or hemolytic icterus a colorless, watery secretion with no biliary pigments or derivatives is collected.

* Described by Sanford.

When 20 c.c. or more of clear duodenal contents is collected it is divided into two 10 c.c. portions in 25 c.c. graduates. To one 10 c.c. portion is added an equal amount, 10 c.c., of a saturated alcoholic solution of zinc acetate (Schlesinger's solution).* The mouth of the graduate is closed by the thumb and the contents thoroughly mixed by vigorous shaking for about one minute. The mixture is then filtered through a single layer of coarse filter paper, the filtrate being collected in another clean, dry graduate. When exactly 10 c.c. of filtrate is obtained it is used for testing for urobilin and urobilinogen. To this mixture, which consists of 5 c.c. of duodenal contents and 5 c.c. of Schlesinger's solution, is added exactly 1 c.c. of Ehrlich's aldehyde reagent† measured with a 1 c.c. pipette. The color of the fluid is usually significant when viewed by transmitted and reflected light. If urobilinogen is present in considerable amount, especially if it predominates, the fluid, on the addition of Ehrlich's reagent, becomes a cherry red, varying in intensity with the amount of chromogen present. When there is a preponderance of urobilin the color by transmitted light is yellow or brown, and by reflected light a green fluorescence characteristic of mixtures of urobilin with zinc salts is noted. The graduate is now set in the dark for fifteen minutes before it is examined spectroscopically. This length of time seems necessary to sufficiently sharpen the absorption bands of the spectrum, while if the mixture stands for longer intervals of time some of the mother substance, urobilinogen, may become converted into urobilin.

While waiting before making the spectroscopic examination the second 10 c.c. portion is tested for bilirubin. To the duodenal contents in the second 25 c.c. graduate is added exactly 10 c.c. of 10 per cent. aqueous solution of calcium chloride made slightly alkaline to litmus with normal sodium hydrate solution. The mixture is thoroughly shaken and then poured into two 15 c.c. centrifuge tubes, 10 c.c. in each. It is then rapidly centrifugalized for a few minutes to collect the precipitate into a compact mass. The supernatant fluid is decanted and the residue washed out of the tubes with a few cubic centimeters of acid alcohol‡ into a porcelain evaporating dish. In all, about 20 c.c. of acid alcohol is used to dissolve the precipitate. The alcohol mixture is carefully heated on a copper warming stage and allowed to boil vigorously. The color, which may be brick red, soon changes to green if there is much bilirubin present. The mixture is concentrated so that its volume just reaches the "U" in an Esbach albuminometer. Alcohol is added to the mark "R." The color of the fluid by transmitted light is then compared with three standard tubes marked +, ++, +++,

* Ethylalcohol, 500 c.c.; zinc acetate, quantity sufficient for saturation.

† Paradimethylaminobenzaldehyde, 4 gm.; hydrochloric acid, 30 c.c.; distilled water, 30 c.c.

‡ Hydrochloric acid, 5 c.c.; alcohol, 20 c.c.

according to the shade of emerald green as viewed by transmitted light. The standard tubes are prepared arbitrarily from specimens containing appreciable amounts, moderately large amounts, and excessive amounts of bilirubin. These alcoholic solutions may be kept indefinitely without change of color, though it may be advisable to place the tube in the dark when it is not in use.

The first mixture is now examined spectroscopically for urobilinogen and urobilin. The spectroscope we use is of the simple students' type having a collimator, with a slit adjustable by a thumb screw, a scale tube, and a draw-tube type of telescope. The light we use is a 250-Watt Tungsten electric lamp mounted on a stand with a green shade reflector. This is adjusted so that when the collimator of the spectroscope is placed about eight inches from the globe a brilliant spectrum is produced. The glare of the light is kept from the eyes by the shade of the lamp, and by a shield of black cardboard perforated so that it may be slipped on the collimator tube. For observing the absorption bands, Schneider uses a 50 c.c. graduated cylinder. Our own observations were made in this manner up to October 1, 1916; since that time we have used a spectrum cell with parallel sides, and of such dimensions that the distance traversed by the rays of light in passing through the fluid is exactly 1 cm. Schneider opens the slit of the collimator eight half-turns or four full turns of the adjusting screw when using a cylinder for examining the solution. We have found this slit too wide with the standard spectrum cell, and have accordingly used a slit of just half the width. Thus to adjust the collimator we completely close the slit and then open it by four half-turns or two complete turns of the adjusting screw. This gives apparently about the same degree of absorption with the standard cell as is obtained with the cylinder when the slit is twice as wide, so that in this way all readings are made to conform to Schneider's standard.

The presence of urobilin is marked by a broad band in the blue end of the spectrum. The violet rays are completely absorbed, and if there is much urobilin present the entire blue portion and nearly all of the green may be obliterated. Urobilinogen absorbs a narrow portion of the spectrum in the yellow at the edge of the green, and if present in large amounts the band may be broad enough to obliterate the entire yellow portion of the spectrum. It is located by its proximity to the "D" Frauenhofer line while urobilin extends from between the "B" and "F" lines to the violet end of the visible spectrum.

The method used by Schneider to estimate the quantity of the absorbing substances is that suggested by Wilbur and Addis. The solution is diluted carefully with alcohol until the absorption bands disappear. The urobilinogen and urobilin differ in their intensity; consequently the disappearance of the absorption bands will occur with different dilutions, although at times the same dilution causes

the clearing of the spectrum in both regions. The end-point is determined when the absorption band disappears, but can be made out faintly when the slit is narrowed to just half of its former opening; that is, when the cylinder method is used the adjusting screw is turned four half-turns. With the standard spectrum cell the end-point is determined by causing the reappearance of absorption bands with two half-turns.

The amount of urobilin and urobilinogen is estimated according to the Wilbur and Addis method for 1000 c.c. by multiplying the number of dilutions by 200. This factor is used since 5 c.c. of duodenal contents is represented in 10 c.c. of filtrate obtained from the mixture with the Schlesinger solution. The number of units of urobilinogen and urobilin are added together and the total number of units reported, *e. g.*, urobilinogen (three dilutions) $3 \times 200 = 600$ units; urobilin (four dilutions) $4 \times 200 = 800$ units; total 1400 units.

Clinical Observations.*

A total of 119 tests have been made in 89 cases. The results will be considered in three groups:

1. Results obtained in a series of miscellaneous cases of which there were 22, and 22 tests.
2. The findings in hemolytic jaundice, 6 cases, 12 tests.
3. The findings in pernicious anemia, 61 cases, 85 tests.

The study includes the tests made in all cases up to November 15, 1916, with the exception of 9 in which the diagnoses were so obscure as to render the results positively and negatively valueless. Brief protocols of the 22 cases in the miscellaneous group are presented.

1. Miscellaneous Cases.

Anemia from Hemorrhage. Case 1 (157200).—Woman, aged fifty years. Uterine myomas. Hysterectomy. Spleen normal in size but hard; liver congested. Hemoglobin, 30 per cent.; red blood cells, 3,470,000.

Duodenal contents: Color, yellow. Urobilin, 200 units; urobilinogen, trace; total, 200+ units.

Case 2 (164791).—Woman, aged forty-one years. Hysterectomy. Appendectomy. Spleen twice normal size; liver slightly enlarged. Hemoglobin, 35 per cent.; red blood cells, 3,190,000.

Duodenal contents: Color, yellow. Urobilin, 200; urobilinogen, 0; total, 200.

Case 3 (156514).—Woman, aged forty-seven years. Cervical polyp. Melancholia of climacteric. Liver normal; spleen normal. Hemoglobin, 50 per cent.; red blood cells, 3,600,000.

Duodenal contents: Urobilin, 200; urobilinogen, 0; total, 200.

* Made by Giffin.

CASE 4 (160583).—Man, aged fifty-nine years. Bleeding hemorrhoids. Liver just palpable; spleen normal. Hemoglobin, 30 per cent.; red blood cells, 3,340,000.

Duodenal contents: Color, yellow. Urobilin, trace; urobilinogen, trace; total less than 200.

CASE 5 (172166).—Woman, aged twenty-five years. Slight menorrhagia. Liver normal; spleen normal. Hemoglobin, 60 per cent.; red blood cells, 4,460,000.

Duodenal contents: Color, yellow. Urobilin, 500; urobilinogen, 0; total, 500.

Chronic Arthritis. CASE 6 (85456).—Man, aged fifty-one years. Mild arthritis and neuralgia. Liver normal; spleen just palpable. Hemoglobin, 72 per cent.; red blood cells, 4,620,000.

Duodenal contents: Color, yellow. Bilirubin trace. Urobilin, 800; urobilinogen, 0; total, 800.

CASE 7 (131120).—Man, aged fifty years. Mild chronic arthritis; dental abscesses; chronic tonsillitis. Liver normal; spleen normal. Possibility of pernicious anemia. Hemoglobin, 38 per cent.; red blood cells, 1,700,000; white blood cells, 12,200; color index, 1.1. (One year ago hemoglobin 57 per cent.; red blood cells 4,080,000.)

Duodenal contents: Urobilin, 600; urobilinogen, 600; total, 1200.

Dental Abscesses; Anemia of Secondary Type. CASE 8 (162929).—Woman, aged thirty-two years. Liver normal; spleen just palpable. Hemoglobin, 52 per cent.; red blood cells, 4,520,000.

Duodenal contents: Color, yellow. Bilirubin trace. Urobilin, 600; urobilinogen, 0; total, 600.

Cholelithiasis. CASE 9 (151433).—Woman, aged fifty-seven years. Gall-stones. Slight possibility of pernicious anemia. Cholecystectomy. Liver normal; spleen normal. Hemoglobin, 49 per cent.; red blood cells, 2,360,000; color index, 1.

Duodenal contents: Color, yellow. Urobilin, 600; urobilinogen, 0; total, 600.

Syphilis. CASE 10 (153153).—Woman, aged thirty-two years. Syphilis of the liver and spleen. Splenectomy, April 29, 1916; spleen 760 grams. Liver very large with gummas and contractures. Hemoglobin, 49 per cent.; red blood cells, 3,430,000.

Duodenal contents: Color, yellow. Urobilin, 1000; urobilinogen, trace; total, 1000+.

CASE 11 (151226).—Woman, aged thirty-four years. Probable luetic anemia. Some evidence of nephritis. Absence of history of pernicious anemia. Liver normal; spleen normal. Hemoglobin, 45 per cent.; red blood cells, 3,330,000; color index, 0.6.

Duodenal contents: Color, yellow. Bilirubin, trace. Urobilin, 800; urobilinogen, 0; total, 800.

Carcinoma. CASE 12 (169462).—Man, aged forty-five years. Carcinoma of stomach. Roentgen findings of extensive carcinoma. Wassermann negative. Hemoglobin, 29 per cent.; red blood cells, 3,190,000.

Duodenal contents: Color, yellow. Urobilin, 400; urobilinogen, trace; total, 400+.

Tuberculosis. CASE 13 (162671).—Woman, aged thirty years. Tuberculous salpingitis. Tuberculous peritonitis found at operation elsewhere. Moderate splenomegaly. Hemoglobin, 35 per cent.; red blood cells, 4,410,000.

Duodenal contents: Color, brown. Bilirubin, ++. Urobilin, 400; urobilinogen, 600; total, 1000.

Chronic Septic Splenomegaly. CASE 14 (154572).—Woman, aged thirty-one years. Chronic septic splenomegaly. History of scarlet fever, frequent sore throat and "la grippe." Attacks of left upper abdominal pain two years. Cesarean section fifteen months previously. Two weeks afterward excruciating upper abdominal pain. Exploration elsewhere; large spleen found but nothing done. History suggestive of abdominal thrombophlebitis. Splenectomy April 12, 1916; spleen, 365 grams. Multiple infarcts. Hemoglobin, 50 per cent.; red blood cells, 4,600,000; white blood cells, 10,400.

Duodenal contents: Color, yellow. Urobilin, 400; urobilinogen, 0; total, 400.

Splenic Anemia. CASE 15 (158085).—Man, aged twenty-nine years. Splenic anemia. History of severe hemorrhages. Melena. Splenectomy; spleen, 780 (?) grams; liver moderately enlarged. Hemoglobin, 45 per cent.; red blood cells, 3,330,000; white blood cells, 3600.

Duodenal contents before splenectomy: Color, yellow. Bilirubin, trace. Urobilin, 1000; urobilinogen, 200; total, 1200.

Portal Atrophic Cirrhosis of Liver. CASE 16 (148570).—Man, aged sixty-two years. Portal atrophic cirrhosis of the liver. History of alcoholism. Ascites. Liver, 720 grams; spleen, 450 grams. Hemoglobin, 70 per cent.; red blood cells, 3,530,000.

Duodenal contents: Color, yellow. Bilirubin, ++. Urobilin, 1000; urobilinogen, trace; total, 1000+.

Polycythemia. CASE 17 (174186).—Man, aged fifty-one years. Polycythemia. Cyanosis. Liver moderately enlarged; spleen moderately enlarged. Diabetes, ten-year history. Nine months previously red blood cell count elsewhere, 9,500,000. Roentgen-ray treatment with improvement. At present hemoglobin, 93 per cent.; red blood cells, 5,320,000. Coagulation time ten minutes (Boggs). Bleeding time, five minutes.

Duodenal contents: Color, brown. Bilirubin, +++. Urobilin, 500; urobilinogen, 500; total, 1000.

Myelogenous Leukemia. CASE 18 (157746).—Man, aged thirty-two years. Spleen enormously enlarged; liver slightly enlarged. Slight degree of jaundice. Duration of history, one and a half years. Splenomegaly one year. Hemoglobin, 45 per cent.; red blood cells, 3,120,000; white blood cells, 496,000; myelocytes, 43.7 per cent.

Duodenal contents: Color, yellow. Urobilin, trace; urobilinogen, trace; total less than 200.

CASE 19 (158647).—Man, aged fifty-two years. Spleen enormously enlarged. Liver normal. Jaundice questionable. Length of history, one year. Splenomegaly, six months. Hemoglobin, 55 per cent.; red blood cells, 3,450,000; white blood cells, 307,000; myelocytes, 29.7 per cent.

Duodenal contents: Color, yellow. Bilirubin, trace. Urobilin, 2000; urobilinogen, 540; total, 2540.

CASE 20 (159989).—Man, aged thirty-nine years. Spleen moderately enlarged; liver normal. Jaundice? Length of history, twenty-two months. Splenomegaly, ten months. Hemoglobin, 53 per cent.; red blood cells, 3,210,000; white blood cells, 341,000; myelocytes, 29 per cent.

Duodenal contents: Color, yellow. Urobilin, 800; urobilinogen, 0; total, 800.

CASE 21 (173747).—Man, aged thirty-nine years. Spleen enormously enlarged; liver moderately enlarged. Slight jaundice. Splenomegaly, two years. Hemoglobin, 42 per cent.; red blood cells, 2,180,000; white blood cells, 8200; myelocytes, 15.7 per cent.

Duodenal contents: Color, brown. Bilirubin, +++. Urobilin, 900; urobilinogen, 200; total, 1100.

Combined Sclerosis. CASE 22 (154069).—Man, aged thirty-eight years. Very ataxic; duration six months. Neurological examination showed findings of advanced combined sclerosis. Wassermann tests negative. Hemoglobin, 54 per cent.; red blood cells, 3,720,000; color index, 0.7. The existence of pernicious anemia is highly probable.

Duodenal contents: Color, brown. Bilirubin, +++. Urobilin, 5500; urobilinogen, 2000; total, 7500.

A summary of the values is given in Table 1.

TABLE 1.—MISCELLANEOUS CASES: SUMMARY OF VALUES.

	Cases.	Urobilin.	Urobilinogen.
Anemia from hemorrhage (averages)	5	275+	0 or trace
Chronic arthritis (averages)	2	700	300
Dental abscesses	1	600	0
Cholelithiasis	1	600	0
Syphilis (averages)	2	900	0 or trace
Carcinoma	1	400	Trace
Tuberculous salpingitis	1	400	600
Chronic septic splenomegaly	1	400	0
Splenic anemia	1	1000	200
Portal atrophic cirrhosis	1	1000	Trace
Polycythemia	1	500	500
Myelogenous leukemia (averages)	4	925+	185
Combined sclerosis (pernicious anemia?)	1	5500	2000

All of these patients save 3 suffered from a moderate or severe anemia. The patient with polycythemia had a hemoglobin of 93

per cent. and a red cell count of 5,320,000 at the time of examination; the value for urobilin was 500 units, and that for urobilinogen 500 units. The patient with portal cirrhosis had a hemoglobin of 70 per cent. and a red cell count of 3,530,000; the urobilin was 1000 units and urobilinogen a trace. One of the patients with chronic infectious arthritis had a hemoglobin of 72 per cent. and a red cell count of 4,620,000; the urobilin was 800 units and urobilinogen zero. These three determinations are unaffected by anemia. They all show total values of approximately 1000 units. It has been concluded by other observers that total values of 1000 units or less are normal.

The values in cases of anemia from hemorrhage were especially low, possibly indicating an actual decrease of blood destruction below normal. In anemias of infectious origin, in syphilis, in carcinoma, and in cirrhosis of the liver the total values for urobilin and urobilinogen were 1000 units or less. Three of 4 patients with myelogenous leukemia gave low determinations; in 1, however, the total was 2540 units.

The group, as a whole, demonstrates consistently low values for the purely secondary types of anemia, irrespective of the severity of the anemia. Patients with simple anemia from hemorrhage present the lowest values of the series.

2. Hemolytic Jaundice.

Twelve determinations of the pigments in the duodenal contents have been made in 6 patients with hemolytic jaundice. Brief protocols of these cases follow:

Case 1 (112836).—Woman, aged forty-nine years. Acquired type of hemolytic jaundice, with the blood picture of a primary anemia. Increased fragility of erythrocytes. Gall-stones and a large slightly cirrhotic liver were found at operation. Weight of spleen, 910 grams. Patient returned during relapse one year and eight and a half months after splenectomy. The liver was then very large. Hemoglobin, 45 per cent.; red blood cells, 3,260,000; white blood cells, 3400; normoblasts, 152 in 300 cells. Duodenal test one year, eight and a half months after splenectomy: Color, brown. Bilirubin, +++. Urobilin, 3000; urobilinogen, 1000; total, 4000. The patient improved markedly after two transfusions, but a subsequent estimation of the pigments was not made.

Case 2 (153245).—Woman, aged thirty-eight years. Severe case of congenital type, with enlarged liver and gall-stones. Increased fragility of erythrocytes. Weight of spleen, 1700 grams. Hemoglobin, 47 per cent.; red blood cells, 2,840,000; white blood cells, 9800; no normoblasts.

Duodenal test one day before splenectomy: Color, yellow. Bilirubin, +++. Urobilin, 4600; urobilinogen, 1000; total, 5600.

Thirty-eight days after splenectomy: Color, yellow. Bilirubin, trace. Urobilin, 1400; urobilinogen, 1800; total, 3200. The hemoglobin at the time of the latter test was 70 per cent.; red cells, 4,960,000; no normoblasts.

CASE 3 (148209).—Man, aged twenty years. Mild case of congenital type. Increased fragility of erythrocytes. At operation the liver showed evidence of early cirrhosis. Weight of spleen, 300 grams. Hemoglobin, 70 per cent.; red blood cells, 4,920,000.

Duodenal test forty-seven days before splenectomy: Color, yellow. Bilirubin, trace. Urobilin, 1400; urobilinogen, 1000; total, 2400. Fourteen days before splenectomy: Color, yellow. Bilirubin, trace. Urobilin, 2000; urobilinogen, 1200; total, 3200. Thirteen days after splenectomy: Color, yellow. Urobilin, 800; urobilinogen, 1000; total, 1800. One hundred and forty days after operation: Color, yellow. Urobilin, trace; urobilinogen, 400; total, 400+.

CASE 4 (161538).—Woman, aged twenty-seven years. Moderately severe case of congenital type. Increased fragility of erythrocytes. Liver probably normal. Gall-stones present. Weight of spleen, 560 grams. Hemoglobin, 64 per cent.; red blood cells, 3,860,000.

Duodenal test three days before splenectomy: Color, yellow. Urobilin, 500; urobilinogen, 500; total, 1000. Twenty-three days after splenectomy: Color, light brown. Bilirubin, trace. Urobilin, 400; urobilinogen, 0; total, 400. At the time of the latter test the hemoglobin was 70 per cent.; red blood cells, 4,680,000.

CASE 5 (162670).—Man, aged thirty-one years. Moderately severe case, probably of congenital type. Increased fragility of erythrocytes. Liver normal. Gall-stones present. Spleen weighed 1250 grams. Hemoglobin 67 per cent.; red blood cells, 3,650,000. Considerable deformity of red cells.

Duodenal test four days before splenectomy: Color, brown. Bilirubin, +++. Urobilin, 1400; urobilinogen, 1800; total, 3200. Twenty-one days after splenectomy: Color, brown. Bilirubin, ++. Urobilin, 1000; urobilinogen, 200; total, 1200. The hemoglobin at the time of the latter test was 80 per cent.; red blood cells, 4,022,000.

CASE 6 (153653).—Man, aged twenty-three years. Mild case with some evidence that a familial factor was present. Increased fragility of erythrocytes. Non-operative. Spleen moderately enlarged. Hemoglobin, 60 per cent.; red blood cells, 3,900,000; white blood cells, 5800; no normoblasts.

Duodenal test: Color, dark yellow. Bilirubin, +. Urobilin, 3000; urobilinogen, trace; total, 3000+. (The low urobilinogen may have been due to delay in making the estimation.)

The values for urobilin and urobilinogen in the duodenal contents are very markedly increased in cases of hemolytic jaundice. A few reported cases have shown even higher values than those we have

demonstrated. High values are found even when a moderate degree of anemia is present. In Case 3 at the time of the first two tests the anemia was not severe; however, abnormally large amounts of pigment were obtained. Severe grades of anemia are associated with very high values; in Case 2, with a red count of 2,840,000, the total values were 5600 units. If these values are a reasonably accurate index of hemolysis, blood destruction in hemolytic jaundice is probably much increased at a time when blood production is not seriously affected. This is in contrast to our experience with pernicious anemia in which the evidence of bone-marrow insufficiency is usually marked and the evidence of blood destruction extremely variable. Patients with pernicious anemia who show high values for urobilin and urobilinogen at a time when the blood count is low frequently show very low values when the blood count has risen to the level of a moderate anemia. Patients with hemolytic jaundice, on the other hand, may show high values with only a slight anemia. In 2 patients with very high values the blood picture simulated that of pernicious anemia. An excessive degree of blood destruction probably exhausted the bone marrow.

In 4 of these patients tested before and after operation there was an appreciable reduction in the values for bile pigments following splenectomy. In 2 of them a decided decrease in the amount of urobilinogen was revealed. In Case 3 a former operation for cholecystitis had not reduced the values to normal. The preoperative values of these 4 patients averaged 2050 units for urobilin and 1100 units for urobilinogen, a total of 3150 units. The average postoperative values at periods varying from thirteen days to four months after splenectomy were 800 units for urobilin and 625 units for urobilinogen, a total of 1425.

TABLE 2.—ESTIMATION OF PIGMENTS IN THE DUODENAL CONTENTS. HEMOLYTIC JAUNDICE.

Case No.	Time before and after splenectomy.	Color.	Bilirubin, units.	Urobilin, units.	Urobilinogen, units.	Total, units.
1 (112836)	1 year 8½ months after	Brown	+++	3000	1000	4000
2 (153245)	1 day before	....	+++	4600	1000	5600
	38 days after	Yellow	Trace	1400	1800	3200
3 (148209)	47 days before	Yellow	Trace	1400	1000	2400
	14 days before	Yellow	Trace	2000	1200	3200
	13 days after	Yellow	0	800	1000	1800
	140 days after	Yellow	0	Trace	400	400+
4 (161538)	3 days before	Yellow	....	500	500	1000
	23 days after	L. brown	Trace	400	0	400
5 (162670)	4 days before	Brown	+++	1400	1800	3200
	21 days after	Brown	++	1000	200	1200
6 (153653)	Non-operative	D. yellow	+	3000	Trace	3000+
Average values before splenectomy			4 cases	2050	1100	3150
Average values after splenectomy			4 cases	800	675	1475

3. Pernicious Anemia.

Eighty-five estimations were made in 61 cases of pernicious anemia. In 6 of the cases the tests were done only after splenectomy, thereby reducing the number of medical and preoperative observations from 61 cases to 55 cases. The average of the readings for urobilin in these 55 cases was 1856.5 units and that for urobilinogen 1604.5 units. The average total for urobilin and urobilinogen was therefore 3461 units. This average is approximately four times normal and remarkably close to the figure obtained by Schneider in his recently reported series.

Nine of the 55 patients showed total values less than 1000; in other words, 84 per cent. gave values of 1000 units and over. It is our experience that patients more than fifty-five years of age, and particularly more than sixty years of age, not uncommonly show low values. In these senile types there is frequently evidence of advanced bone-marrow damage with little active hemolysis. Some of these anemias may, in reality, be osteosclerotic in origin, but this distinction is difficult to make clinically.

It is also our experience that as the blood improves in pernicious anemia the duodenal values quickly decline. When the red cells reach 3,500,000 the duodenal values are quite apt to run below 1000 units total. The average in 3 patients with red cell counts above 3,500,000 cells was 433.3 units for urobilin and 466.6 units for urobilinogen, making a total of 899.9 units. Moreover, a few patients who have had repeated tests during treatment have shown this same rapid decline in values when the anemia became of moderate grade. Hemolytic jaundice, on the other hand, gave high values even when the anemia was slight.

It is therefore to be concluded that a certain number of patients with undoubted pernicious anemia do show low total values at certain times. On the other hand, urobilinogen will be present in an appreciable amount. The most noteworthy constant is the presence of an estimable amount of urobilinogen. Urobilinogen was absent in only 1 of the 55 medical and preoperative cases; in 2 others there was a trace; in 52 urobilinogen was present in relatively large amounts; in 24, or nearly one-half of the cases, urobilinogen was present in even larger amounts than urobilin. The presence of large quantities of urobilinogen before splenectomy and its complete absence in at least 75 per cent. of the cases after splenectomy are very striking findings and may have an important significance.

The highest total values were obtained in patients with red cell counts between 2.5 and 3.5 million cells. They were slightly lower in patients with erythrocyte counts between 1.5 and 2.5 million cells and considerably lower in patients with erythrocyte counts below 1.5 million cells. The lowest values were obtained in patients

with counts over 3.5 million cells, but even in these urobilinogen was present in a distinctly abnormal amount. The most active hemolysis seems to occur in the patients with counts between 2.5 and 3.5 million cells, but this may be due to the fact that patients are only rarely seen at the onset of a period of active blood destruction. As a group these latter cases show evidence of very active hemolysis and at the same time evidence of active blood production.

TABLE 3.—ESTIMATION OF PIGMENTS IN THE DUODENAL CONTENTS: VALUES WITH RESPECT TO ERYTHROCYTE COUNT.

PERNICIOUS ANEMIA (MEDICAL AND PREOPERATIVE).

	Number of estimations.	Average urobilin, units.	Average urobilinogen, units.	Total, units.
Erythrocytes 1.5 million and below	11	1496.3	1472.7	2969.0
Erythrocytes 1.5 to 2.5 million .	32	1864.3	1425.0	3589.3
Erythrocytes 2.5 to 3.5 million .	13	2346.1	1653.8	3999.9
Erythrocytes 3.5 million and over	3	433.3	466.6	899.9
HEMOLYTIC JAUNDICE (MEDICAL AND PREOPERATIVE.)				
Erythrocytes 3.5 million and over	4	1650.0	850.0	2500.0

Age. The average totals for patients under fifty-five years of age showed very little variation by decades. Over the age of fifty-five years there was a decided drop both in urobilin and urobilinogen. Between the ages of fifty-six and sixty years the totals average 2644.2; over the age of sixty years they average 1600, while under fifty-five years the average totals for decades vary between 3325 and 4238.

TABLE 4.—ESTIMATION OF PIGMENTS IN THE DUODENAL CONTENTS: VALUES WITH RESPECT TO AGE.

PERNICIOUS ANEMIA (MEDICAL AND PREOPERATIVE).

Age of patient.	Number of cases.	Average urobilin, units.	Average urobilinogen, units.	Total, units.
30 years and under .	3	2266.6	1833.3	4099.9
31 to 40	10	1955.0	1370.0	3325.0
41 to 50	12	2075.0	1691.6	3766.6
51 to 55	16	2200.6	2037.5	4238.1
56 to 60	9	1372.0	1272.2	2644.2
Over 60 years . . .	5	660.0	940.0	1600.0

Size of Spleen. High values were obtained both in patients with large spleens and in those with small spleens. Lower values were obtained in those with spleens of moderate size, that is, weighing from 200 to 500 gm. The highest urobilinogen values were obtained in patients with spleens of 200 gm. and less. It is impos-

sible to determine the significance of these findings with respect to the size of the spleen. The degree of pathological or of functional damage in the liver may be the important factor. Patients with small spleens have more constantly shown evidence of advanced pathological change in the liver.

TABLE 5.—ESTIMATION OF PIGMENTS IN THE DUODENAL CONTENTS: VALUES WITH RESPECT TO WEIGHT OF SPLEEN.

PERNICIOUS ANEMIA (PREOPERATIVE ESTIMATIONS).

Weight of spleen.	Number of cases.	Average urobilin, units.	Average urobilinogen, units.	Total, units.
200 grams and below .	4	3000.0	2000.0	5000.0
200 to 500 grams . .	12	2342.5	1487.5	3830.0
Over 500 grams . .	6	3233.3	1433.3	4666.6

VALUES AFTER SPLENECTOMY.

A very striking reduction is observed in the values after splenectomy. A total of 19 patients was examined after splenectomy; 13 both before and after operation. The average of the total values for these 13 patients became reduced from 4492.2 to 1134.6 units. Urobilinogen in 10 of the 13 cases was reduced to zero or a trace. Of the entire group of 19 examined after splenectomy, 13 gave values for urobilinogen of zero or a trace. Three showed urobilinogen over 1000 units after splenectomy, but judging from the clinical history these readings probably became reduced later.

Two patients who had high values for urobilinogen following splenectomy, showed no definite improvement in the anemia. The liver was enlarged in each instance and an exposure of the liver to radium was suggested. The application of 50 mg. of radium over five areas for a total of ten hours was followed by a very marked reduction in the size of the liver, a fall in duodenal values and a prompt improvement in the anemia.

TABLE 6.—ESTIMATION OF PIGMENTS IN THE DUODENAL CONTENTS: PREOPERATIVE AND POSTOPERATIVE VALUES.

	Number of cases.	Average urobilin, units.	Average urobilinogen, units.	Total, units.
Pernicious anemia (medical and preoperative)	55	1856.5	1604.5	3461.0
Pernicious anemia (preoperative) .	13	2970.7	1521.5	4492.2
Pernicious anemia (postoperative)	13	815.4	319.2	1134.6
Pernicious anemia (postoperative)	10 of 13	480.0	0 or trace	480.0+
Hemolytic jaundice (preoperative)	4	2050.0	1100.0	3150.0
Hemolytic jaundice (postoperative)	4	800.0	675.0	1475.0

TABLE 7.—ESTIMATION OF PIGMENTS IN THE DUODENAL CONTENTS. PERNICIOUS ANEMIA (SPLENECTOMY).

Case No.	Date of splenectomy.	Time before and after splenectomy.	Color.	Bilirubin, units.		Urobilin, units.		Urobilinogen, units.		Total, units.		Red blood cells, millions.	Weight of spleen, grams.
				Before operation.	After operation.	Before operation.	After operation.	Before operation.	After operation.	Before operation.	After operation.		
1 (152922)	Mar. 10, 1916	7 days before	....	+++	....	2000	....	600	....	2600	....	1.96	150
		21 days after	Yellow	....	+	....	1400	....	0	....	1400	1.48	
2 (152671)	15, 1916	20 days before	....	....	....	5000	....	1000	....	6000	....	2.94	508
		13 days before	....	....	....	6000	....	4000	....	10000	....		
		21 days after	....	....	....	....	800	....	0	....	800	4.40	
3 (154493)	25, 1916	9 days before	Brown	+	....	3000	....	2000	....	5000	....	2.34	525
		17 days after	Yellow	....	++	....	200	....	0	....	200	1.89	
4 (153395)	29, 1916	9 days before	Brown	+++	....	3500	....	Trace	....	3500+	....	2.53	450
		24 days after	Yellow	....	Trace	....	Trace	....	Trace	....	200−	3.14	
5 (154299)	April 8, 1916	9 days before	Yellow	+++	....	4000	....	2000	....	6000	....	2.80	133
		21 days after	Brown	....	++	....	6800	....	1400	....	8200	2.73	
		4 mos. after	Brown	....	+++	....	1800	....	1400	....	3200	2.16	
6 (157146)	20, 1916	3 days before	Brown	+++	....	3200	....	2600	....	5800	....	2.96	270
		19 days after	Brown	....	....	....	1400	....	Trace	....	1400+	3.25	
7 (151021)	May 4, 1916	5 days before	Yellow	....	....	5400	....	4600	....	10000	....	2.46	111
		27 days after	Yellow	....	Trace	....	200	....	0	....	200	1.96	
8 (157663)	June 2, 1916	1 mo. before	Brown	+	....	840	....	760	....	1600	....	2.41	410
		14 days before	Yellow	....	+	500	....	600	....	1100	....	2.22	
		2 mos. after	Yellow	....	+	....	400	....	0	....	400	3.49	
9 (157290)	5, 1916	2 mos. before	Yellow	....	....	600	....	800	....	1400	....	1.66	192
		18 days after	Yellow	....	Trace	....	Trace	....	Trace	....	200−	5.20	
10 (161677)	15, 1916	6 days before	Yellow	+	....	400	....	400	....	800	....	2.72	770
		36 days after	Yellow	....	++	....	Trace	....	0	....	200−	3.00	
11 (160970)	27, 1916	11 days before	Brown	+++	....	800	....	200	....	1000	....	3.33	300
		29 days after	Yellow	....	Trace	....	Trace	....	0	....	200−	4.38	
12 (158136)	28, 1916	2 mos. before	Brown	+++	....	2500	....	2000	....	4500	....	1.51	300
		7 days before	Brown	+++	....	600	....	600	....	1200	....	1.94	
		23 days after	Yellow	....	++	....	800	....	1400	....	2200	1.80	
13 (170115)	Oct. 3, 1916	8 days before	Brown	+++	....	8000	....	2000	....	10000	....	2.35	600
		1 mo. after	Brown	....	+++	....	800	....	1300	....	2100	2.65	
		2 mos. after	Yellow	....	+	....	600	....	800	....	1400	3.14	

Transfusions.

Transfusions did not seem to affect the duodenal values. However, estimations were not made the first or second day following transfusion, at which time an increase in the amounts of pigments might be expected. Estimations made five and ten days following transfusion gave no unusual values.

Bilirubin.

An excess of bilirubin was usually present when large amounts of urobilin and urobilinogen were found. This relationship was by no means constant. An excess of bilirubin was not infrequently found with low values for urobilin and urobilinogen, and very small amounts of bilirubin were occasionally found with high values. The amount of bilirubin therefore does not run parallel with the values for urobilin and urobilinogen. These values are probably indicative of some quite different function or impairment of function in pathological conditions.

Summary.

1. With a few slight modifications of technic we have used the method of Schneider in estimating quantitatively the amounts of urobilinogen and urobilin in the duodenal contents obtained by means of an Einhorn tube. The procedures are simple and can be carried out in any clinical laboratory. The results are comparable with those obtained by the more complicated and time-consuming methods in which stool extracts are used for the estimation of these pigments.

2. In a group of 22 miscellaneous cases, low values were obtained in patients with anemia from hemorrhage, carcinoma, tuberculous peritonitis, syphilis, portal cirrhosis, chronic infectious arthritis, and gall-stones. They were low in 3 of 4 patients with myelocytic leukemia. The amounts of these pigments were especially low in cases of anemia from hemorrhage.

3. In hemolytic jaundice the values were consistently high even when severe anemia was not present. The values fell appreciably after splenectomy, but not as promptly as in pernicious anemia.

4. In pernicious anemia the amounts of urobilin and urobilinogen in the duodenal contents were above normal in 84 per cent. of the cases. The amount of urobilinogen was constantly increased when the anemia was severe. Patients over the age of fifty-five showed lower values than younger patients. The values presented no definite relationship to the size of the spleen. Following splenectomy there was a very definite decrease in the amounts of urobilin and urobilinogen; the decrease in urobilinogen was especially noticeable.

5. The amounts of bilirubin in the duodenal contents did not run constantly parallel to the amounts of urobilin and urobilinogen.

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PLEURAL EOSINOPHILIA.

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REVIEWING 2758 articles for his monograph on eosinophilia, E. Schwarz,[1] in 1914, was able to find but 68 cases of pleural eosinophilia, although this condition had been described by Widal and Ravaut[2] as early as 1900. Bayne-Jones[3] believes the phenomenon occurs more frequently than this would seem to indicate, and attributes the small number of reported cases to the fact that many pleural fluids have not been examined microscopically and that relatively few have been stained with polychrome dyes. According to Bayne-Jones, pleural eosinophilia occurs in about 1 to 5 per cent. of all cases of pleural effusion, as determined by investigators applying constant methods to cases in series.

In many cases of primary pleurisy, when the fluid is examined on the first day of its appearance, a very slight, transient eosinophilia is noted.[4] This eosinophilia vanishes rapidly before the onrush of the neutrophiles. Again during convalescence, eosinophiles may be encountered in small numbers. In these cases, however, the percentage of eosinophiles seldom reaches and does not exceed 5 per cent., in marked contrast to the so-called group of true pleural eosinophilias where these cells range from 10 per cent. upward, and

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